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99. Evaluation of 215 Candidate Fungicides for Use in Fish Culture

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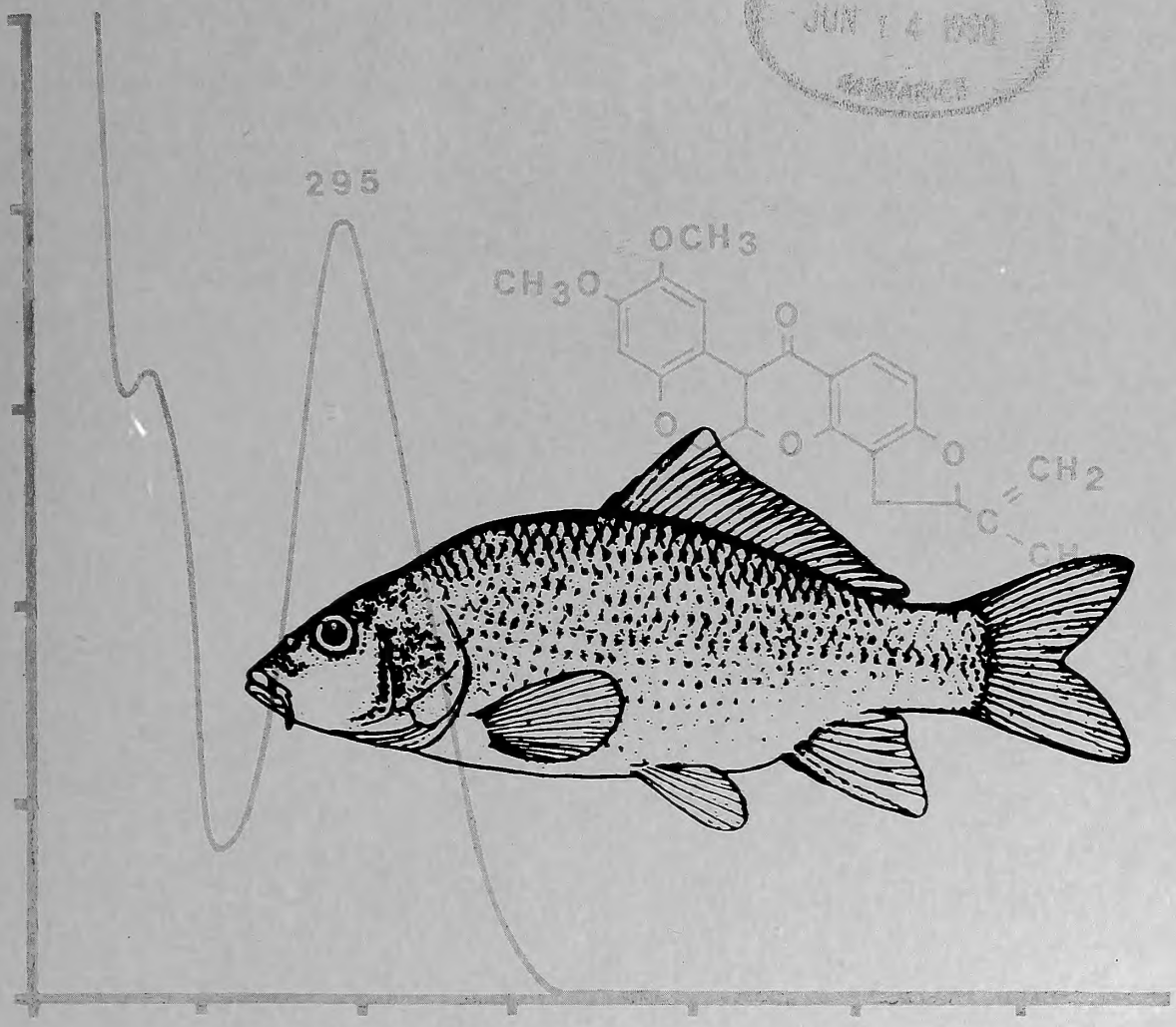

UNITED STATES DEPARTMENT OF THE INTERIOR
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Evaluation of 215 Candidate Fungicides for Use in Fish Culture

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ABSTRACT.—The fungicidal activity of 215 compounds was evaluated by comparing their effectiveness with that of malachite green. More than half were found to be unsuitable as aquatic fungicides in preliminary screening tests because of their lack of activity against fungi, toxicity to fish or eggs, insolubility in water, or potential carcinogenicity. After further testing, 30 compounds were selected for evaluation of their activity against fungi on eggs of rainbow trout (*Oncorhynchus mykiss*). Four compounds—8-quinolinol, 8-quinolinol sulfate, dichlorophen, and formalin—inhibited fungal growths on dead and live fish eggs and demonstrated the greatest potential as replacements for malachite green. The quinolinols cannot be considered for use as aquatic antifungal agents, however, because they were toxic to eggs at efficacious concentrations (≥1.0 mg/L). Dichlorophen, at concentrations of 5.0 mg/L, was effective for eggs of channel catfish (*Ictalurus punctatus*) that had been separated, but was toxic for eggs clustered in the original egg mass; it showed potential for use on channel catfish eggs at 2.5 mg/L for 15 min, but was only marginally effective against fungi on rainbow trout eggs. Formalin controlled fungal infections on rainbow trout eggs at a concentration of 250 mg/L for 60 min—a concentration substantially lower than has been previously reported.

Fishes are commonly parasitized by various species of aquatic fungi that cause fungal infections or saprolegniasis; several species of the pathogenic fungi may occur together on a single infected fish. Saprolegniasis is usually considered to be secondary to bacterial or viral infections (Richards 1977; Richards and Pickering 1978), but there is evidence that some of the Saprolegniales may act as primary pathogens (Neish 1977; Richards and Pickering 1978). Fungal infections often follow physical damage to the surface of fish (Roberts and Shepherd 1974; Richards and Pickering 1978) caused by handling and intensive culture conditions. In the wild, fungal infections develop in abrasions received during territorial defense, redd digging, or spawning (White 1975; Richards and Pickering 1978).

Fungal infections of hatchery-reared fish can usually be controlled with formalin (Neish and Hughes 1980; Bailey 1984) or malachite green (Meyer and Hoffman 1976; Bailey 1983a). Formalin is the only registered aquatic fungicide, but it is generally not completely effective on adult fish. The antifungal activity of malachite green is extremely high, but it is unlikely to be approved (registered) by the U.S. Food and Drug Administration (Schnick and Meyer 1978) because of its potential teratogenicity (Meyer and Jorgenson 1983). Its use is now limited to the treatment of nonfood fish (e.g., eggs or adult salmon held for spawning) under an Investigational New Animal Drug Application held by the U.S. Fish and Wildlife Service.

Because of the status of these two fungicides and the substantial monetary losses that result from fungal infections in fish hatcheries, fish culturists need an effective registered fungicide to replace malachite green. Our purpose here is to report on the efficacies of a number of potential fungicides against three species of aquatic fungi and to evaluate their potential for approval and future use on fish and incubating fish eggs.

Materials and Methods

Pure strains of aquatic fungi were obtained from the American Type Culture Collection (ATCC). *Saprolegnia hypogyna* (ATCC 28275) and *Achlya flagellata* (ATCC 14566) were used for in vitro testing (preliminary screening and determination of minimum inhibitory concentration) and *Saprolegnia ferax* (ATCC 36146) was used to infect eggs of rainbow trout (*Onchorhynchus mykiss*) for confirmatory and in vivo tests.

Test procedures used were those developed by Bailey (1983a,b; 1984). The method involves an in vitro screening technique modified from that of Golden and Oster (1947) and a minimum inhibitory concentration determination based on the percent inhibition of growth in diameter of colonies. Cultures of fungi on agar were exposed to five concentrations of each candidate compound for 15 and 60 min.

An egg to agar transfer test was conducted to establish the concentrations that would control fungal growth on a natural substrate (dead rainbow trout eggs) for 48 h (Bailey 1984). Dead (cold shocked) rainbow trout eggs were exposed to chemicals at concentrations of 10 to 100 times their minimum inhibitory concentrations. The egg to agar transfer provides a confirmatory test and is representative of the in vivo activity of a compound. Levels of inhibition are the concentrations that prevent growth of fungi on either the egg surface or on agar.

The most promising candidate compounds were tested on incubating (green) rainbow trout eggs received from Trout Lodge, McMillin, Washington, and Erwin National Fish Hatchery, Tennessee. Duplicate groups of 500 eggs were artificially infected with *S. ferax* and exposed to candidate fungicides in Heath incubator trays for 15 or 60 min. Treatments were administered three times weekly for 2 weeks. Two control groups of eggs were used for each study: one was uninfected with fungi and untreated with fungicide; the other was infected with fungi but untreated. Upon completion of the testing, the degree of fungal infection and the number of eggs that hatched were analyzed to determine the efficacy of each chemical. The data were then compared to those for malachite green, the reference compound. A chi-square test ($\alpha = 0.05$, df = 1) was used to determine whether treatments with the candidate fungicides caused significant differences in rates of infection and hatching.

Results

A total of 215 compounds were tested to determine antifungal activity against the three pure-strain species of pathogenic aquatic fungi. Of these, 120 were considered unsuitable as aquatic fungicides because they were inactive against the fungi, toxic to fish or eggs, insoluble in water, or potentially carcinogenic (Table 1; Tables 1–6 follow the text). Although four—copper-8-quinolinolate, crystal violet, Du-Ter, and Phaltan—were highly effective against the fungi, all were toxic to fish and fish eggs at the effective levels or were potentially teratogenic (e.g., crystal violet, a chemical homologue of malachite green). The minimum inhibitory concentrations of candidate compounds were based on fungal growth (colony diameter) and categorized in three levels of activity (Tables 2–4): high (≤10 mg/L), moderate (>10 to 100 mg/L), or low (>100 mg/L).

Before in vivo testing, the most promising compounds were evaluated further in confirmatory tests (Table 5). Only four of the candidate compounds adequately inhibited fungal growth on incubating rainbow trout eggs in in vivo tests (Table 6): dichlorophen (2.0 mg/L), formalin (250 mg/L), 8-quinolinol (30.0 mg/L), and 8-quinolinol sulfate (35.0 mg/L).

The proportions of rainbow trout eggs infected were all significantly lower than in the untreated controls after 15-min exposures to dichlorophen (45%, $\chi^2 = 13.3$), formalin (37%, $\chi^2 = 30.4$), 8-quinolinol (83%, $\chi^2 = 146.2$), and 8-quinolinol sulfate (20%, $\chi^2 = 40.8$). Likewise, inhibition after 60-min exposures was significant (dichlorophen: 48%, $\chi^2 = 7.8$; formalin: 15%, $\chi^2 = 261.9$; 8-quinolinol: 30%, $\chi^2 = 24.8$; 8-quinolinol sulfate: 20%, $\chi^2 = 28.3$). Hatching success was highest after treatments with dichlorophen and 8-quinolinol sulfate (Figure).

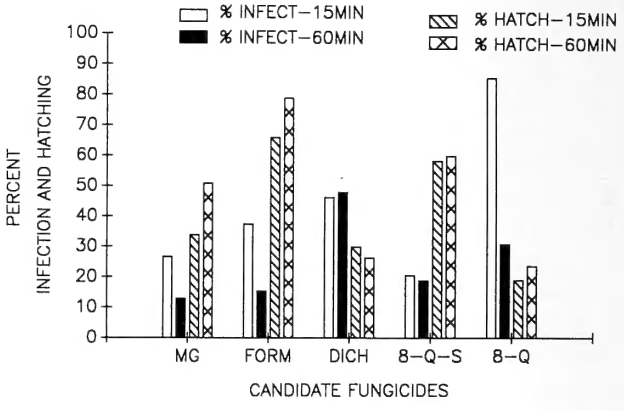

Figure. Relative percent fungal infection of rainbow trout eggs after treatment with candidate fungicides and resulting hatching success. MG = malachite green; FORM = formalin; DICH = dichlorophen; 8-Q-S = 8-quinolinol sulfate; and 8-Q = 8-quinolinol.

Channel catfish eggs that had been separated and exposed to 5.0 mg/L dichlorophen for 15 min showed only 2% fungal infection, and hatching success was about 91%. When eggs were exposed to 2.5 mg/L dichlorophen, the fungal infection rate rose to 5.0% but hatching rate for the uninfected eggs improved to 96%.

Eggs of channel catfish (*Ictalurus punctatus*), which were allowed to remain in the egg mass, were exposed to 5.0 mg/L and 2.5 mg/L dichlorophen. Treatment with 5.0 mg/L dichlorophen killed 98% of the eggs; however, treatment with 2.5 mg/L dichlorophen resulted in 20% infection and 88% hatching success.

Of the candidate fungicides used in 60-min exposures, formalin at 250 mg/L provided the best control of fungal growth on rainbow trout eggs (only 15% infected) and yielded an acceptable level of hatching (>65%) after a 15- or 60-min exposure (Figure). In order of decreasing efficacy, the compounds ranked as follows: formalin > 8-quinolinol sulfate > dichlorophen > 8-quinolinol.

Discussion

In laboratory tests, the powerful antifungal agent malachite green was 5 to 30 times more effective than all except 5 of the 215 candidate compounds: copper-8-quinolinolate, crystal violet, Du-Ter (47%), Phaltan, and 8-quinolinol. Malachite green thus provides an excellent reference standard with which candidate fungicides can be compared. The registration of malachite green is unlikely, although limited use of the compound might be acceptable if suitable filtration units are used (Marking et al. 1989).

The 8-quinolinol compounds controlled fungi in situ; however, the parent compound and the water soluble salts had detrimental side effects: At efficacious concentrations, 8-quinolinol reduced the hatching to unacceptable levels and 8-quinolinol sulfate caused posthatching mortality.

Dichlorophen, an anthelmintic (Shah et al. 1984), has shown some potential for controlling pathogenic fungi (Mussa and Russell 1977; Alderman and Polglase 1984) and protozoans (Takeuchi et al. 1985; Griffin 1989). Alderman and Polglase (1984) reported that the sodium salt of dichlorophen is effective in vitro against fungi at 1.0 mg/L. We did not test the sodium salt of dichlorophen, but the minimum inhibitory concentration for the phenol form of the compound was 7.5 mg/L. Perhaps water solubility enhances the toxicity.

In an unpublished report on preliminary tests with channel catfish eggs, B. R. Griffin[1] noted that 15-min immersions in 5 or 10 mg/L of dichlorophen for 4 consecutive days arrested the spread of fungal growth, and resulted in no evidence of pre- or posthatching toxicity. The results of our tests of separated channel catfish eggs exposed to dichlorophen generally agreed with these observations. In contrast, dichlorophen was extremely toxic when we used it on eggs clustered in the egg mass.

In in vivo tests with fungused rainbow trout eggs, dichlorophen was only marginally effective. In preliminary toxicity tests, groups of rainbow trout eggs were subjected to 15-min dips in solutions of dichlorophen for 4 consecutive days. Within 48 h after treatments, mortality was high. Dichlorophen is also toxic to fish. The LC50 values for channel catfish and rainbow trout were 8.2 and 3.0 mg/L in 1-h exposures and 0.9 and 0.5 mg/L in 3-h exposures.

It is clear from data shown here that formalin was superior to any of the candidate fungicides screened. Unfortunately, hatchery managers have not fully exploited its effectiveness. Formalin continues to show excellent antifungal activity against natural fungal infections caused by the Saprolegniales. The safe, controlled use of formalin should help control fungal problems until better fungicides can be developed.

Our data showed that dichlorophen may have potential as an antifungal for use on channel catfish eggs; however, its usefulness on fungused rainbow trout eggs is still questionable. The toxicity of quinolinols to fish and to fish eggs will probably eliminate them from further consideration as replacements for malachite green.

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Table 1. *Chemicals that were ineffective against Saprolegniales, toxic to nontarget organisms, or otherwise considered unsuitable for further testing.*

A-23 (Abbott Laboratories)	Copper carbonate	Kanamycin sulfate[a]
A-48 (Abbott Laboratories)	Copper sulfate (basic)[a]	Kasumin (50%)
A-49 (Abbott Laboratories)	Copper sulfate (tri-basic)[a]	Metalaxyl
A-56 (Abbott Laboratories)	m-Coumaric acid	Methyl orange
A-150A (Lilly Laboratories)	o-Coumaric acid	Methylene blue
A56620 (Abbott Laboratories)	*p*-Coumaric acid	Morestan
Acriflavin	Coumarin	Nalidixic acid (Na^+ salt)
7-Amino-4-methylcoumarin	Coumarin 35	OAC-3289[a]
7-Amino-4-trifluoromethylcoumarin	Coumarin 138	O-Phenylphenol (Dowcide A)
2-Aminopyridine	Coumarin 152	O-Phenylphenol (Dowcide 1)
Ascorbic acid	Coumarin 153	Oxolinic acid (20%)[a]
Baycor	Coumarin 311	Phaltan 50%
Bayleton	Cuprimyxin (0.5%)[a]	2-Phenoxyethanol
Baytan	Cutrine-plus	Piperalin
BayVP2674	Disulfiram	Plantvax
Benomyl	Dithane M-45 (Mancozeb)	Polyoxin AL (filtered)
Benzalkonium chloride[a]	Dithane Z-78 (Zineb)	Polyoxin AL (suspension)
Boric acid	EDTA (disodium)	Polyoxin Z (filtered)
Busan 85	Fast green	Polyoxin Z (suspension)
Busan 881	5-Fluorocytosine	Potable aqua
Captan[b]	Formalin[a]	Potassium chloride
Captan 50W[b]	Fosfomycin disodium salt	Potassium iodide
Carbenicillin disodium salt	Fumagillin	Potassium sorbate
Catch and Release[a]	Funginex	Previcur-N[a]
Catechol	Griseofulvin	Quinoline hydrochloride
Cellulase (pH 7.69)	Halox E-100[a]	Quinoline QL
Citcop-4E	2-Hydroxy-5-nitropyridine[a]	Quinolinol HCl
Clotrimazole	Ipronidazole[a]	2-Quinolinol
CO (tech) (Derse & Schroeder)	Ipropran	6-Quinolinol

Table 1. *Continued.*

7-Quinolinol	Roccal (tech)	Tannic acid
8-Quinolinol benzoate	Ronilan	Terrazole
8-Quinolinol (free base)	Salicylanilide I	Thuringiensin (3%)
4-Quinolinol-2-carboxylic acid	Small Fish Saver[a]	Topsin M
8-Quinolinol-5-sulfonic acid	Sodium chloride	1-2-4-Triazole
8-Quinolinol potassium sulfate	Sodium iodide	Trifluralin
4-Quinolinol-trihydrate	Sodium sulfate	Ultramarine blue CP-18
8-Quinolyl acetate	Sodium sulfite	Ultramarine blue FP-64
R07-4488/006[b]	Streptomycin SO_4	Vancide 51
Ridomil	T204C (Tavolek Company)	Vangard 10W
Roccal II (50%)	Tamed iodine	Vitavax

[a]Antifungal activity less than desirable, but screened in further tests because of water solubility or current registrations.
[b]Rejected because of toxicity or carcinogenicity.

Table 2. *Minimum inhibitory concentrations (mg/L) of candidate aquatic fungicides that showed high antifungal activity against* Achlya flagellata *and* Saprolegnia hypogyna *in 15- and 60-min exposures.*

	Achlya flagellata		*Saprolegnia hypogyna*	
Chemical	15 min	60 min	15 min	60 min
AL-BB-001	5.0	8.0	5.0	5.0
Alpha-terthienyl	—	1.0	—	1.0
Amical 48	5.0	3.0	3.0	0.75
ρ-Benzoquinone	30.0	10.0	10.0	10.0
Busan 30	5.0	3.0	5.0	3.0
Busan 30L	10.0	10.0	10.0	10.0
Busan 30WB	10.0	10.0	10.0	10.0
Busan 1030	10.0	10.0	10.0	10.0
Copper oxychloride sulfate	>10<100	10.0	10.0	10.0
Copper-8-quinolinolate	1.0	1.0	1.0	1.0
Crystal violet	1.0	0.3	0.75	0.5
Dichlorophen	10.0	7.5	7.5	7.5
Du-Ter (47.5%)	1.0	1.0	1.0	1.0
Du-Ter (tech)	3.0	3.0	1.0	1.0
Dyrene	30.0	10.0	10.0	10.0
Herbisan 5	10.0	10.0	10.0	10.0
5-Hydroxy-1,4-naphthoquinone	10.0	6.0	6.0	6.0
8-Hydroxy-5-nitroquinoline	30.0	9.0	3.0	9.0
Malachite green	1.0	1.0	0.5	0.5
Malachite green (carbinol)	<10	<10	<10	<10
Malachite green (neutralized)	<10	<10	<10	<10
Phaltan (tech)	1.0	1.0	1.0	1.0
Polyphase 17WD	5.0	3.0	3.0	1.0
Polyphase AF-1	3.0	3.0	3.0	1.0
Polyphase P-100	5.0	5.0	3.0	1.0
8-Quinolinol	1.0	7.5	1.0	1.0
8-Quinolinol copper salt	3.0	3.0	3.0	1.0
8-Quinolinol HCl	5.0	>15<30	5.0	5.0
V-101 (Abbott Laboratories)	10.0	5.0	3.0	1.0
V-102 (Abbott Laboratories)	30.0	10.0	5.0	3.0

Table 3. *Minimum inhibitory concentrations (mg/L) of candidate aquatic fungicides that showed moderate antifungal activity in 15- and 60-min exposures.*

	Achlya flagellata		*Saprolegnia hypogyna*	
Chemical	15 min	60 min	15 min	60 min
Actidione	30.0	30.0	>30	>30
AO (tech) (Derse & Schroeder)	15.0	3.0	40.0	15.0
Bayer 73	25.0	15.0	30.0	30.0
Black algaetrine	>100	75.0	50.0	10.0
Blasticidin-S	500	500	30	3.0
Botran	75.0	75.0	75.0	50.0
Cellulase (pH 5.02)	>100	>10	>100	>10
Chlorazene (powder)	50.0	30.0	75.0	30.0
Chlorazene (tablets)	>100	>10<100	>10<100	>10<100
Copper sulfate (tri-basic)	100	100	100	100
Defungit	125	100	125	100
5,7-Dichloro-8-quinolinol	>100	>10<100	>10<100	>10<100
Dithane M-22 (Maneb)	50.0	75.0	50.0	50.0
Dithianon	700	75.0	300	100
Dodine	30.0	30.0	75.0	75.0
Ethyl violet	15.0	15.0	15.0	15.0
Hyamine 3500	500	100	500	100
2-Hydroxy-1,4-naphthoquinone	75.0	45.0	75.0	45.0
Irumamycin	>100	>10<100	>10<100	>10<100
Karathane	100	100	100	30.0
Lesan (technical)	60.0	60.0	60.0	36.0
Miconazole	300	75.0	75.0	30.0
5-Nitro-8-quinolinol	24.0	8.0	8.0	24.0
5-Nitrosalicylic acid	112.5	75.0	112.5	45.0
Polyalkylene glycol-iodine	100	100	150	75.0
Potassium permanganate	210	80.0	210	80.0
8-Quinolinol sulfate	100	100	300	100
TFM (36.3%)	75.0	50.0	75.0	10.0
TFM (85.6%)	30.0	30.0	30.0	30.0
TFM (tech)	30.0	30.0	30.0	30.0
2-(4-Thiazolyl) benzimidazole	30.0	30.0	30.0	30.0
Thiram	100	75.0	30.0	30.0
Thiram 42S	>100	>100	>10<100	10.0

Table 4. *Minimum inhibitory concentrations (mg/L) of candidate aquatic fungicides that showed low antifungal activity in 15- and 60-min exposures.*

	Achlya flagellata		*Saprolegnia hypogyna*	
Chemical	15 min	60 min	15 min	60 min
Amobam	>100	>100	>100	>100
Amphotericin B-Type I	>1,000	>1,000	>1,000	>1,000
BAS-389-01F	500	500	300	300
Benzalkonium chloride	500	300	500	300
Catch and Release	>1,000	>1,000	>1,000	>1,000
CGA-64251	1,000	500	500	300
Copper sulfate (basic)	300	100	100	100
Copper sulfate (crystals)	300	300	300	300
Cuprimyxin (0.5%)	2,500	750	875	750
Diquat dibromide	5,000	2,500	30.0	30.0
Enilconazole	750	300	300	100
Formalin	700	200	700	200
Halox E-100	>1,000	750	>1,000	>1,000
2-Hydroxy-5-nitropyridine	>1,000	>1,000	>1,000	>1,000
Ipronidazole	>1,000	>1,000	>1,000	>1,000
Kanamycin sulfate	>1,000	>1,000	1,000	1,000
Ketoconazole	300	300	300	100
Lesan 70% WP	>1,000	>1,000	100	100
Leuco-malachite green	800	800	>800	>800
Naftifine	>100	>100	>100	>100
Nalidixic acid (free acid)	500	500	500	750
OAC-3289	—	—	>1,000	>1,000
Oxolinic acid (20%)	>1,000	>1,000	>1,000	>1,000
Polyram 80% (filtered)	1,000	750	750	500
Polyram 80% (suspension)	500	500	500	500
Previcur-N	>1,000	>1,000	>1,000	>1,000
8-Quinolinol citrate	>100	>100	>100	>100
Small Fish Saver	1,000	750	750	500
Sodium omadine	>100	>100	>100	>100
Solricin 135	250	250	250	250
Surflan	200	150	200	150
Thiabendazole	1,000	1,000	300	300

Table 5. *Concentrations (mg/L) of fungicides that were effective for the control of aquatic fungi on dead rainbow trout eggs in egg to agar transfer tests.*

	Incubation time			
	24 h		168 h	
Chemical	15 min	60 min	15 min	60 min
Actidione	100	100	100	100
AL-BB-001 (40%)	—[a]	—	—	—
Amical 48	>250	25	>250	25
p-Benzoquinone	>100	>100	>100	>100
Black algaetrine	—	—	—	—
Busan 30	75	50	75	50
Busan 30L	10	10	>100	100
Busan 1030	75	75	>100	100
Busan 30WB	100	75	100	100
Chloramine-T	—	—	—	—
Copper-8-quinolinolate	>10	>10	>10	>10
Defungit	1,000	—	3,000	3,000
Dichlorophen	10.0	10.0	>100	100
Dithianon	>300	>300	>300	>300
Dodine	188	188	188	188
Du-Ter (47.5%)	—	—	—	—
Du-Ter (tech)	75	75	>2,000	>2,000
Formalin	—	—	—	—
5-Hydroxy-1,4-naphthoquinone	—	—	—	—
LD (Alcide Corp.)	—	—	—	—
Lesan (70%)	300	100	>1,000	750
Malachite green	8.0	8.0	>80	>80
5-Nitro-8-quinolinol	—	—	—	—
Phaltan (technial)	—	—	—	—
Polyphase 17WD	>100	10	>100	10
Polyphase P-100	30	10	50	10
Potassium permanganate	—	—	—	—
8-Quinolinol	100	100	300	100
8-Quinolinol copper salt	50	50	>50	>50
8-Quinolinol sulfate	—	—	—	—
TFM (36.3%)	150	250	375	250
TFM (85.6%)	100	100	300	300
TFM (tech)	100	100	100	100
Thiabendazole	>1,000	>1,000	>1,000	>1,000
Thiram 42S	—	—	—	—
V-101 (Abbott Laboratories)	—	—	—	—
V-102 (Abbott Laboratories)	—	—	—	—

[a] Dashes indicate chemicals tested in in vivo tests, but not in egg to agar transfer tests.

Table 6. *Evaluation of candidate fungicides selected for in vivo tests with artificially infected live rainbow trout eggs.*

Chemical	Concentration (mg/L)	Effect on eggs
Actidione	25	Toxic
AL-BB-001 (40%)	10	Toxic
Alcide	1:10:1	Toxic
Amical 48	25	Toxic
p-Benzoquinone	10	Toxic
Black algaetrine	30	Toxic
Busan 30WB	1	Toxic
Chloramine-T	75	Toxic
Copper-8-quinolinolate	20	Toxic
Defungit	50	Ineffective
Dichlorophen	2	Marginally effective
Dithianon	100	Toxic
Dodine	75	Toxic
Du-Ter (tech)	1	Toxic
Du-Ter (47.5%)	1	Toxic
Formalin	250	Effective
5-Hydroxy-1,4-naphthoquinone	10	Highly toxic
LD (Alcide Corp.)	1:10:1	Toxic
Malachite green	5	Effective[a]
5-Nitro-8-quinolinol	15	Toxic
Phaltan (technial)	5	Toxic
Polyphase 17WD	10	Toxic
Polyphase P-100	10	Toxic
Potassium permanganate	50	Marginally effective
8-Quinolinol	30	Effective
8-Quinolinol copper salt	1	Toxic
8-Quinolinol sulfate	35	Effective[b]
TFM (36.3%)	15	Toxic
Thiabendazole	30	Toxic
Thiram 42 S	30	Toxic
V-101 (Abbott Laboratories)	15	Toxic
V-102 (Abbott Laboratories)	5	Toxic

[a] Potential risk to human health.
[b] Post-hatch mortality.

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The fungicidal activity of 215 compounds was evaluated by comparing their effectiveness with that of malachite green. More than half were found to be unsuitable as aquatic fungicides in preliminary screening tests because of their lack of activity against fungi, toxicity to fish or eggs, insolubility in water, or potential carcinogenicity. Four compounds—8-quinolinol, 8-quinolinol sulfate, dichlorophen, and formalin—inhibited fungal growths on dead and live fish eggs and demonstrated the greatest potential as replacements for malachite green. The quinolinols are questionable for use as aquatic fungicides because of their toxicity. Dichlorophen holds some promise for use against fungus on eggs of channel catfish (*Ictalurus punctatus*), but formalin was effective at a concentration of 250 mg/L—substantially lower than has been previously reported.

Key words: Fungicides, dichlorophen, formalin, 8-quinolinol, fish eggs.

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